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Metabolism of Acetate, Methanol, and Methylated Amines in Intertidal Sediments of Lowes Cove, Maine†

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The fates and the rates of metabolism of acetate, trimethylamine, methylamine, and methanol were examined to determine the significance of these compounds as in situ methane precursors in surface sediments of an intertidal zone in Maine. Concentrations of these potential methane precursors were generally <3 μM, with the exception of sediments containing fragments of the seaweed *Ascophyllum nodosum*, in which acetate was 96 μM. [2-^{14}C]acetate turnover in all samples was rapid (turnover time <2 h), with $^{14}CO_2$ as the primary product. [^{14}C]trimethylamine and methylamine turnover times were slower (>8 h) and were characterized by formation of both $^{14}CH_4$ and $^{14}CO_2$. Ratios of $^{14}CH_4/^{14}CO_2$ from [^{14}C]trimethylamine and methylamine in uninhibited sediments indicated that a significant fraction of these substrates were catabolized via a non-methanogenic process. Data from inhibition experiments involving sodium molybdate and 2-bromoethanesulfonic acid supported this interpretation. [^{14}C]methanol was oxidized relatively slowly compared with the other substrates and was catabolized mainly to $^{14}CO_2$. Results from experiments with molybdate and 2-bromoethanesulfonic acid suggested that methanol was oxidized primarily through sulfate reduction. In Lowes Cove sediments, trimethylamine accounted for 35.1 to 61.1% of total methane production.

In marine sediments, competition for substrate has been considered a major factor limiting methanogenesis (1–3, 7–9, 14, 15, 18, 22, 25). Sulfate-reducing bacteria (SRB) out-compete methane-producing bacteria (MPB) for both H_2 and acetate over a wide range of sulfate concentrations because thermodynamic and kinetic considerations favor sulfate reduction (10, 10a, 21). Studies of competition for acetate have indicated that acetate hydrolysis to CO_2 and CH_4 occurs only in sulfate-depleted sediments and, by inference, that the reduction of CO_2 by H_2 is a significant source of methane in marine sediments (2, 9, 14, 18, 20, 25). Even though SRB have a competitive advantage, H_2 uptake by MPB is apparently not entirely excluded.

Most studies of marine sediments have not considered methane production from substrates other than H_2 or acetate. However, Senior et al. (22) have suggested that some unknown substrate(s) may be more important than H_2 in marsh sediments. Oremland et al. (16) and Oremland and Polcin (17) have proposed that trimethylamine (TMA) and methanol are noncompetitive methane precursors in salt marsh soils and San Francisco Bay sediments. Observations by Oremland et al. (16) indicate that TMA and methanol metabolism could account for measured rates of methane production. Winfrey and Ward (26) have also noted that methylamine (MA) is readily converted to methane and that the metabolism of this compound may be of greater importance than H_2 for methanogenesis. The metabolism of methylated amines in marine sediments could be especially significant because high concentrations of these compounds are produced by marine organisms for osmoregulation (28).

We report here a study of acetate, methanol, MA, and TMA metabolism at in situ concentrations in surface sediments of an intertidal mud flat in Maine. Results indicate that acetate and methanol were catabolized primarily by SRB, that TMA and MA may have been degraded by both SRB and MPB, and that TMA was a significant source of methane (35 to 61%).

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MATERIALS AND METHODS

Sediments were obtained from the intertidal zone of Lowes Cove, Maine (43°56'N, 69°35'W). Tides in this region are semidiurnal, with an amplitude of 2 to 3 m and a salinity of 30 ppt (30 ‰). The intertidal sediments of Lowes Cove consist of fine silts and clays with an organic content of 1 to 2%. Aspects of the sediment chemistry and biology have been described elsewhere (P. T. Rahaim, M.S. thesis, University of Maine, Orono, 1980).

Surface sediments (0 to 10 cm depth) of two types were collected from the midcove region. In one type, designated here as bare sediments (BS), the upper 5 cm appeared oxidized, and the lower 5 cm was more reduced, as indicated by the presence of iron sulfides. BS were also characterized by low dissolved sulfide (<10 μM) and relatively low rates of sulfate reduction (<100 nmol/cm^3 per day) and methanogenesis (Table 1; G. M. King, unpublished data). Little or no sulfate depletion was observed over the upper 10 cm in BS. The second sediment type, designated as *Ascophyllum nodosum* sediments (ANS), was obtained from shallow depressions within the BS. These depressions usually contained a lens of water (<10 cm depth) at low slack tide and partially buried fragments of the rockweed *A. nodosum*. ANS were visibly black at the sediment surface and were often colonized by sulfide-oxidizing bacteria which formed a white mat on the surface. Dissolved sulfide (~1 mM) and rates of sulfate reduction (>1 μmol/cm^3 per day) and methanogenesis were relatively high at ANS sites (Table 1; King, unpublished data). Sulfate concentrations in ANS were generally less than in BS but were still ~15 mM at 10 cm. BS were characterized by a diverse fauna consisting of a variety of decapods and molluscs; this faunal assemblege was notably absent from ANS sites (D. S. Shaw, unpublished data).

Surface sediments from BS and ANS sites were collected by using 10-cm inside diameter coring tubes. Cores were placed in an anaerobic glove bag, and the upper 10 cm of the sediment was removed and slurried (1:1) with 1-μm-filtered, deoxygenated seawater (30 ‰). This treatment would have resulted in little or no increase in sulfate concentrations in BS and only moderate increases in ANS. Because only moderate, if any, sulfate depletion was observed in these sediments, it was presumed that no mixing of sulfate-reducing and methanogenic layers occurred. Aliquots (10 cm^3) of the slurry were dispensed into 15-ml Vacutainer tubes or Hungate pressure tubes (Bellco Glass, Inc.) which were subsequently flushed with O_2-free 100% N_2 and sealed. Then 0.3 ml of a solution containing one of the following substrates was injected by needle and syringe into each of 15 replicate tubes containing BS or ANS: [^{14}C]TMA, ~0.4 μCi/ml (5.0 mCi/mmol; New England Nuclear); [^{14}C]MA, ~0.3 μCi/ml (51.8 mCi/mmol; New England Nuclear); [^{14}C]methanol, ~0.2 μCi/ml (56.9 mCi/mmol; Amersham); [2-^{14}C]acetate, ~2 μCi/ml (51 mCi/mmol, New England Nuclear). Triplicate tubes of BS and ANS containing the first three of the above-mentioned solutions were incubated at 20°C (field temperature) for 0, 1, 3, 6, or 12 h. Triplicate tubes, as described above, containing [2-^{14}C]acetate were incubated at 20°C for 0, 0.5, 1, 2, or 4 h. Reactions were terminated by injecting with a needle and syringe 1 ml of a 20% solution of glutaraldehyde.

Total rates of methane production for the ANS and BS slurries were measured by using quadruplicates of each sediment type to which no radioisotopes were added. A 0.5-ml volume of the gas phase of each of these control samples was analyzed for methane by using a Varian 1440 flame ionization gas chromatograph (Varian Instruments, Inc.) fitted with a stainless steel column (0.32 cm outer diameter by 1 m) containing Porpak Q (Waters Associates, Inc.). The column was operated at 75°C with a carrier gas of N_2 at 30 ml/min. Control sediments were incubated for 12 h at 20°C for rate determinations.

In similar experiments, 10 cm^3 of a 1:1 slurry of ANS was preincubated for 24 h with sodium molybdate (20 mM final concentration) or 2-bromoethanesulfonic acid (BES; 27 mM final concentration) to ensure effectiveness of the inhibitors. Afterwards, 0.3 ml of the above-mentioned solutions of radioisotopes was injected by needle and syringe. Three replicates of each substrate plus inhibitors were incubated at 20°C for 6 h. Activity was terminated as described above.

Production of $^{14}CH_4$ and $^{14}CO_2$ was determined by subsampling the gas phases of the tubes with a needle and syringe. Samples (1.0 ml) were analyzed with a Varian 3760 gas chromatograph fitted with a stainless steel column (0.32 cm outer diameter by 1 m) containing Porpak N (Waters). The column was operated at 40°C with a flow rate of N_2 of 20 ml/min. $^{14}CH_4$ and $^{14}CO_2$ were eluted with retention times of 1.3 and 4 min, respectively, and were passed through a flame ionization detector to oxidize $^{14}CH_4$. Exhaust from the detector was bubbled through a series of two scintillation vials containing 7 ml of 0.5 N KOH to trap $^{14}CO_2$ originally in the sample and $^{14}CO_2$ originating from any $^{14}CH_4$. A separate set of traps was used for $^{14}CH_4$ and $^{14}CO_2$; trap sets were selected by using a switching valve. A 7-ml volume of Aqueous Counting Scintillant (Amersham Searle) was added to each vial, and radioactivity was determined by using a Beckman LS 7500 scintillation counter (Beckman Instruments, Inc.). Recovery of label in the traps was >80%. The distribution of $^{14}CO_2$ and $H^{14}CO_3^-$ between the gas and dissolved phases of the sediment samples was determined by adding known amounts of $H^{14}CO_3^-$ to tubes as described above and then measuring $^{14}CO_2$ in the headspace after an 8-h equilibration period. The observed distribution of $^{14}CO_2$ and $H^{14}CO_3^-$ was used to correct headspace $^{14}CO_2$ for total $^{14}CO_2$.

Acetate uptake in ANS and BS was determined by measuring the activity of the [2-^{14}C]acetate remaining in samples to which [2-^{14}C]acetate had been added. A 1.0-ml volume of the interstitial water from each of the sediment samples was acidified to remove $^{14}CO_2$ and then was mixed with 3 ml of Aqueous Counting Scintillant for liquid scintillation counting. Previous studies involving radio-gas chromatography as described by Lovley and Klug (11) indicated that added acetate was converted only to gaseous end products and not to other soluble metabolites.

The recovery of added label in the preceding experiments averaged >95% for the various substrates in both ANS and BS. Recovery was determined from the sum of $^{14}CO_2$, $^{14}CH_4$, and dissolved ^{14}C measured at the final incubation point. No $^{14}CO_2$ or $^{14}CH_4$ was measured for controls containing glutaraldehyde.

The potential response of ANS to substrate additions was determined by incubating 50 cm^3 of a 1:1 slurry of the 0- to 10-cm depth interval in 300-ml

biological oxygen demand bottles containing an atmosphere of O_2-free 100% N_2. Sediments were incubated in the dark for 48 h at 20°C. Basal rates of methane production during this period were determined by removing samples (0.5 ml) of the gas phase of the bottles and analyzing methane with the Varian 1440 flame ionization detector previously described. After 48 h, solutions of TMA, MA, methanol, or acetate were added to a final concentration of 1 mM. Rates of methane production were determined for triplicates of each substrate during an additional 48-h incubation period at 20°C.

Concentrations of methanol and methylamines were determined by gas chromatography, using a Varian 3740 with flame ionization detectors. Interstitial water from both ANS and BS was collected by centrifugation and was shipped frozen to the Kellogg Biological Station, Hickory Corners, Mich., for analysis (12). Briefly, methanol was determined after distillation and injection of the distillate onto a 2-m glass column (2-mm inside diameter) containing Porpak Q. Recovery of added methanol was 73%. MAs were analyzed by evaporating to dryness aliquots of acidified interstitial water containing 20 mg of $(NH_4)_2SO_4$. A volume of 10 N NaOH (10 ml) was injected into sealed bottles containing the residue from evaporation. MAs in the headspaces of the bottles were assayed by injection of 1 ml of the headspace gas into a 2-m glass column (2-mm inside diameter) containing Carbopak B–4% Carbowax 20M–0.8% KOH (Supelco, Inc.) operated at 85°C with helium as a carrier. Recoveries of MAs were approximately 70%.

Acetate concentrations in interstitial water were measured at the I. C. Darling Center, Walpole, Maine, using a Varian 3760 gas chromatograph. Aliquots of interstitial water (1 to 2 ml) were acidified (pH <2) with H_3PO_4 and distilled under vacuum (23). Distillate was injected onto a 1-m glass column (4-mm inside diameter) containing Carbopak B–4% Carbowax 20M–10% H_3PO_4 (Supelco). Absolute recovery of acetate was >85%, but concentrations were determined relative to an internal standard, isovaleric acid, the recovery of which also exceeded 85%.

RESULTS

The time course of $^{14}CO_2$ formation from [2-^{14}C]acetate was hyperbolic (Fig. 1) for both ANS and BS. Added [2-^{14}C]acetate was converted primarily to $^{14}CO_2$. About 92 and 46% of the added acetate was recovered as $^{14}CO_2$ within 4 h in ANS and BS, respectively. These relative differences in $^{14}CO_2$ production were evident in rates of [2-^{14}C]acetate uptake (Fig. 1). Decreases in [2-^{14}C]acetate were approximately mirrored by increases in $^{14}CO_2$ production, and turnover times calculated from either acetate uptake or end product formation were similar. Acetate turnover times based on $^{14}CO_2$ production at 1 h were 1.8 and 2.9 h for ANS and BS, respectively.

The time courses of $^{14}CH_4$ and $^{14}CO_2$ from both [^{14}C]TMA and [^{14}C]MA were also hyperbolic (Fig. 2 [BS data not shown]). The percent catabolism of these substrates in ANS was less than that of acetate but was similar to acetate

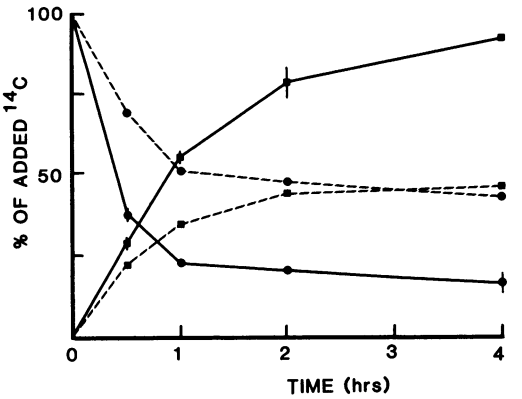

FIG. 1. Uptake of [2-^{14}C]acetate and production of $^{14}CO_2$ in slurries of ANS and BS. Each point is the mean of triplicate samples. Representative standard error bars are shown for ANS (± one standard error); error bars smaller than the symbols are not shown. Symbols: ●, [2-^{14}C]acetate; ■, $^{14}CO_2$; —, ANS; ----, BS.

catabolism in BS. Average total turnover times over the 0- to 6-h incubation period were 8.5 and 9.0 h for TMA and MA respectively, in ANS, and 12.6 and 8.7 h, respectively, in BS. Ratios of $^{14}CH_4/^{14}CO_2$ at the termination of the experiments were relatively low (≤2) for [^{14}C]TMA and [^{14}C]MA in both sediment types (Table 1).

End product formation from [^{14}C]methanol differed somewhat from that of other substrates. Both $^{14}CH_4$ and $^{14}CO_2$ were produced rapidly, within 1 to 3 h, but no significant changes were observed thereafter (Fig. 2). Turnover times to $^{14}CH_4$ were long relative to other substrates

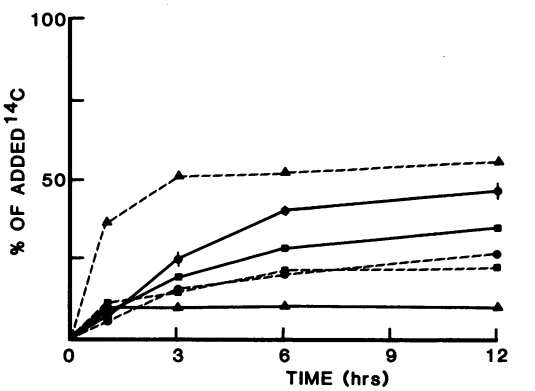

FIG. 2. Production of $^{14}CO_2$ and $^{14}CH_4$ from [^{14}C]methanol, [^{14}C]TMA, and [^{14}C]MA. Each point is the mean of triplicate samples of ANS slurries. Representative standard error bars are shown for products from [^{14}C]TMA (± one standard error); error bars smaller than the symbols are not shown. Symbols: ▲, products of [^{14}C]methanol; ●, products of [^{14}C]TMA; ■, products of [^{14}C]MA; —, $^{14}CH_4$; ----, $^{14}CO_2$.

TABLE 1. Methane production and patterns of substrate concentrations and metabolism in slurries of Lowes Cove sediments

Site	Substrate	Substrate concn[a]	Methane production[b]	$^{14}CH_4/^{14}CO_2$ ratio[c]	% of total methane production[d]
BS	Acetate	11.0	<0.001	<0.003	0.5
	Methanol	3.3	0.003	0.01	2.4
	TMA	2.2	0.036	1.95	61.1
	MA	ND	0.028	0.51	
ANS	Acetate	96.0	<0.001	<0.001	5.5
	Methanol	1.5	<0.001	0.19	0.1
	TMA	2.4	0.091	2.04	35.1
	MA	ND	0.057	1.34	

[a] Expressed as micromoles per liter of the sediment slurries described in the text. ND, Not detected.

[b] Methane production is expressed as the fraction of ^{14}C substrates converted to $^{14}CH_4$ and $^{14}CO_2$ via methanogenesis per hour. Values were determined by using measured $^{14}CH_4$ and $^{14}CO_2$ calculated from theoretical stoichiometries for each substrate; values were calculated for the 0- to 6-h incubation period.

[c] Ratio of $^{14}CH_4/^{14}CO_2$ after 6 h of incubation.

[d] Average rates ($n = 4$) of total methane production in BS were 0.29 nmol/cm^3 per h; average rates ($n = 4$) in ANS were 1.4 nmol/cm^3 per h.

(Table 1), though total turnover times were less than for [^{14}C]TMA and [^{14}C]MA. Ratios of $^{14}CH_4/^{14}CO_2$ from [^{14}C]methanol were quite low (Table 1), and there was no trend for values increasing with time.

Concentrations of the various substrates investigated were all relatively low, with the exception of acetate in ANS (Table 1). MA was not detectable in BS or ANS. The observed concentrations and rates of $^{14}CH_4$ production were used to calculate the contribution of each substrate to total methane production measured for the ANS and BS slurries. Methane production from acetate and methanol was negligible in both sediment types (Table 1). Actual contributions from MA could not be calculated because MA was not detected; potential contributions based on the lower limit of detection, 1 μM, were <10%. The percentage of total methane production derived from TMA varied from 35.1% in ANS to 61.1% in BS.

Addition of sodium molybdate (20 mM final concentration) resulted in an almost complete inhibition of acetate oxidation and an increase in the ratio of $^{14}CH_4/^{14}CO_2$ from [2-^{14}C]acetate; the total fraction of acetate catabolized was ~1% (Table 2). $^{14}CH_4/^{14}CO_2$ ratios from [^{14}C]TMA, [^{14}C]MA, and [^{14}C]methanol also increased markedly in the presence of molybdate, as did the total fraction of substrates catabolized. The addition of BES (27 mM final concentration) resulted in a significant decrease in $^{14}CH_4$ formation and $^{14}CH_4/^{14}CO_2$ ratios from all substrates (Table 2). With the exception of a significant decrease in the fraction of [^{14}C]TMA catabolized, total substrate catabolism was similar in sediment with or without BES (Tables 1 and 2). Total methane production increased in molybdate-amended sediments (5.1 nmol/cm^3 per h) relative to unamended controls (0.92 nmol/cm^3 per h) and decreased upon addition of BES (0.75 nmol/cm^3 per h). During the course of the inhibition experiments, total methane production was decreased by only 18% in the presence of 27 mM BES. Subsequent experiments indicated that longer incubation periods (up to 48 h) were necessary to achieve complete inhibition of methanogenesis by BES.

The addition to ANS of substrates at 1 mM concentrations resulted in increased methane production during a 48-h incubation (Table 3). The greatest overall stimulation resulted from amendments with TMA, followed by MA and then acetate. Incubation with methanol resulted in no notable stimulation (Table 3). Stimulation of methane production by the above-mentioned substrates was measurable within 6 h of incubation. Similar results were obtained when BS were used, though rates of methane production were lower.

DISCUSSION

In Lowes Cove surface sediments, as in other marine sediments, sulfate reduction greatly exceeded methanogenesis. Rates of sulfate reduction for the 0- to 10-cm depth interval in Lowes Cove during summer months were typically 2 orders of magnitude higher than methane production (>100 nmol of sulfate reduced per cm^3 per day versus <7 nmol of CH_4 produced per

TABLE 2. Effects of selective inhibitors on substrate metabolism in ANS slurries

Substrate	Inhibitor	% Added substrate metabolized[a]	$^{14}CH_4/^{14}CO_2$ ratio
Acetate	Na_2MoO_4 (20 mM)	1.0	0.4
TMA		91.7	2.8
MA		83.2	2.1
Methanol		71.8	1.8
Acetate	BES (27 mM)	66.5	
TMA		6.0	0.2
MA		48.8	<0.01
Methanol		75.4	

[a] Fraction of label recovered as $^{14}CH_4 + ^{14}CO_2$ after 6 h.

TABLE 3. Effect of substrate additions on rate of methanogenesis in ANS[a]

Substrate	Rate 48 h before addition	Rate 48 h after addition	% Change
None	1.3 (0.5)	2.0 (2.9)	53.8
TMA	1.1 (0.3)	45.7 (16.6)	4,054.5
MA	0.7 (0.5)	8.9 (5.8)	1,171.4
Methanol	1.6 (0.5)	1.4 (0.6)	−12.5
Acetate	1.1 (0.3)	2.7 (0.6)	145.5

[a] Final concentration of all substrates was 1 mM. Rates are expressed as nanomoles of methane produced per cubic centimeter of sediment per hour. Values represent the mean of three samples; standard errors are in parentheses.

cm^3 per day in BS). The predominance of sulfate reduction was evident from the almost complete oxidation of [2-^{14}C]acetate to $^{14}CO_2$ (Fig. 1; Table 1). Inhibition of sulfate reduction in ANS by molybdate resulted in increased formation of $^{14}CH_4$ from [2-^{14}C]acetate; however, acetate was not a significant methane precursor. Similar observations have been reported for intertidal and subtidal coastal sediments as well as salt marsh soils (2, 7, 9, 14, 18, 20, 22).

Methanol was also an unimportant substrate for methanogenesis in surface sediments of Lowes Cove. Though some $^{14}CH_4$ was formed from [^{14}C]methanol, the contribution of methanol to total methanogenesis was ~2% (Table 1). The lack of significant methanogenesis from methanol at in situ concentrations or 1 mM additions may have been due to competition by SRB. Ratios of $^{14}CH_4/^{14}CO_2$ were <0.2 (Table 1), significantly lower than the ratio of 3 expected for utilization of methanogens alone (13). Addition of molybdate to ANS resulted in a significant increase in $^{14}CH_4/^{14}CO_2$ ratios (Table 2), which suggested oxidation of methanol by SRB. In view of reports that SRB can use methanol as an energy source (19), it is not surprising to find significant methanol oxidation by sulfate reduction in marine sediments.

In freshwater sediments with low sulfate concentrations, methanol appeared to be metabolized primarily by MPB, presumably as a result of the greater competitive ability of methanogens in freshwater systems (12). In the only other reports of methanol metabolism in marine sediments, Oremland et al. (16) and Oremland and Polcin (17) indicated that methanogenesis was stimulated by 10 mM methanol; however, Oremland et al. (16) and Oremland and Polcin (17) also indicated that methanol was apparently not used by SRB at similar concentrations. Further studies are needed to establish the conditions under which methanol may be a significant source of methane or substrate for sulfate reduction. Differences among sediments with respect to sources of methanol, such as pectins, should receive particular attention.

Because MA concentrations were not detectable, MA presumably accounted for a negligible fraction of total methane production, even though a significant fraction of added [^{14}C]MA was converted to $^{14}CH_4$. Potential MA metabolism, like that of methanol, appeared to be the result of both sulfate reduction and methanogenesis, as indicated by $^{14}CH_4/^{14}CO_2$ ratios and results of experiments with molybdate and BES (Tables 1 and 2). Data from Lowes Cove intertidal sediments were consistent with reports of MA metabolism in other intertidal sediments (27). Unlike methanol, however, MA additions (1 mM final concentration) resulted in an immediate and marked stimulation of methanogenesis (Table 3), suggesting that populations of MPB in Lowes Cove are better adapted to compete for MA than for methanol.

Of the varied substrates examined, TMA accounted for the greatest fraction of total methane production (Fig. 2; Table 1). In BS where organic matter is relatively low, TMA accounted for 61% of total production, whereas in ANS, TMA accounted for somewhat less. The difference between these two sediments may have resulted from a greater availability of H_2 in the ANS due to the incorporation of significant quantities of *A. nodosum* organic matter into the sediment.

Oremland et al. (16) have noted that TMA turnover could account for 90% of the methane production in slurries of a salt marsh soil amended with *Spartina foliosa*. The greater importance of TMA in marsh soils than in Lowes Cove may be the result of greater concentrations and production of TMA in marshes. Methylated amines are found in high concentrations in halophytes such as *Spartina* and may represent readily available sources of TMA (4, 6, 24; G. M. King, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I166, p. 167). Comparisons of TMA metabolism among sediments must therefore take into consideration variability in TMA sources if differences are to be understood or explained.

[^{14}C]TMA metabolism in both ANS and BS resulted in higher $^{14}CO_2$ formation than expected from uptake by methanogens alone; incubation of sediments with molybdate yielded ratios more consistent with methanogenesis (Tables 1 and 2). These data suggested that TMA utilization resulted from both sulfate-reducing and methanogenic activity. However, it should be noted that TMA metabolism has not been demonstrated for cultures of sulfate reducers. SRB have been reported to metabolize choline, a common methylated amine (5, 19). Studies with cell-free extracts and cultures demonstrated that acetate and TMA were formed from choline, but

further metabolism of TMA by sulfate reducers has not been described (5, 19).

In summary, neither acetate, methanol, nor methylamine was a significant methane precursor in Lowes Cove. Acetate and methanol metabolism at in situ concentrations were characterized by sulfate reduction, whereas that of MA appeared to result from both sulfate-reducing and methanogenic activity. However, methane production from MA was insignificant because concentrations were not detectable. TMA utilization at in situ concentrations accounted for 35 to 61% of total methane production. The fraction of methane derived from TMA appeared to depend in part on sediment organic matter concentrations. Inhibition experiments with molybdate and BES suggested that TMA was also oxidized by a process other than methanogenesis and that in contrast with previous reports (16, 17), competition between sulfate reducers and methanogens may include methanol and methylated amines as well as H_2 and acetate.

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